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Tyrosine Phosphorylation Regulates the SH3-mediated Binding of the Wiskott-Aldrich Syndrome Protein to PSTPIP, a Cytoskeletal-associated Protein*

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Wiskott-Aldrich syndrome is an X-linked hematopoietic disease that manifests itself in platelet deficiency and a compromised immune system. Analysis of hematopoietic cells from affected individuals reveals that mutations in the Wiskott-Aldrich syndrome protein (WASP) result in structural and functional abnormalities in the cell cortex, consistent with the suggestion that WASP is involved with regulation of the actin-rich cortical cytoskeleton. Here we report that WASP interacts with a recently described cytoskeletal-associated protein, PSTPIP, a molecule that is related to the *Schizosaccharomyces pombe* cleavage furrow regulatory protein, CDC15p. This association is mediated by an interaction between the PSTPIP SH3 domain and two polyproline-rich regions in WASP. Co-expression of PSTPIP with WASP *in vivo* results in a loss of WASP-induced actin bundling activity and co-localization of the two proteins, which requires the PSTPIP SH3 domain. Analysis of tyrosine phosphorylation of PSTPIP reveals that two sites are modified in response to v-Src co-transfection or pervanadate incubation. One of these tyrosines is found in the SH3 domain poly-proline recognition site, and mutation of this tyrosine to aspartate or glutamate to mimic this phosphorylation state results in a loss of WASP binding *in vitro* and a dissolution of co-localization *in vivo*. In addition, PSTPIP that is tyrosine phosphorylated in the SH3 domain interacts poorly with WASP *in vitro*. These data suggest that the PSTPIP and WASP interaction is regulated by tyrosine phosphorylation of the PSTPIP SH3 domain, and this binding event may control aspects of the actin cytoskeleton.

PSTPIP is a recently described coiled-coil and SH3 domain-containing protein that is homologous to CDC15p, a *Schizosaccharomyces pombe* phosphoprotein involved with the assembly of the cytokinetic cleavage furrow (1–3). PSTPIP is associated with protein-tyrosine phosphatase (PTP)[1] HSCF, a member of the PEST family of PTPs (4), via a critical tryptophan in the PSTPIP coiled-coil domain (2), and this association mediates the dephosphorylation of tyrosine residues in PSTPIP that are modified either by co-expression of the v-Src tyrosine kinase or in the presence of the PTP inhibitor pervanadate. Throughout interphase, the coiled-coil domain localizes PSTPIP to actin-rich regions of the cell, including the cortical cytoskeleton and lammelipodia, and the protein migrates to the cleavage furrow during cytokinesis. Overexpression of PSTPIP in mammalian cells induces filapodial extension and cell rounding, and induced expression of the mammalian protein in *S. pombe* results in a dominant negative inhibition of cytokinesis (1). These data suggest that PSTPIP is a tyrosine phosphorylated cytoskeletal regulatory protein that may be involved with the control of cytokinesis.

Wiskott-Aldrich syndrome is an X-linked hematopoietic disease that presents with platelet abnormalities and immunodeficiency (5, 6), and mutations in the Wiskott-Aldrich syndrome protein (WASP) cause this syndrome (7–9). WASP is a multi-domain protein containing pleckstrin homology, CDC42-binding, polyproline, and actin regulatory motifs (7). Hematopoietic cells from Wiskott-Aldrich syndrome patients have abnormalities in their cortical cytoskeletons (6, 10), and overexpression of WASP induces bundling of F actin, which may be regulated by CDC42, a GTPase involved with cytoskeletal modulation (11, 12). A WASP homologue in *Saccharomyces cerevisiae* is involved with cortical actin assembly and cytokinesis (13, 14). In addition, WASP binds to various tyrosine kinases as well as the Nck and GRB 2 adaptor proteins, suggesting a link with cellular signal transduction pathways (15–19). These data suggest that WASP is a scaffold that mediates the assembly of various signaling and structural components involved with cytoskeletal regulation.

Together, these data suggest the involvement of both PSTPIP and WASP in the control of the cytoskeleton. Here we show a physical interaction between the SH3 domain of PSTPIP and proline-rich regions of WASP. This interaction appears to modulate the actin bundling activity localized to the C terminus of WASP (11). Analysis of the tyrosine phosphorylation of PSTPIP reveals that one of the two phosphotyrosines in this protein is within the polyproline-binding pocket of the SH3 domain, and this modification appears to regulate the binding between PSTPIP and WASP. This study describes a novel mechanism for the modulation of the interaction between two components involved with regulation of the cytoskeleton.

MATERIALS AND METHODS

Two-hybrid Screening—Yeast two-hybrid screening was performed as described previously (1). Briefly, full-length PSTPIP (1) was cloned in frame with the galactose DNA-binding domain and used as bait to screen a library derived from murine BaF3 hematopoietic progenitor cells. Yeast capable of growing in the absence of histidine were used as sources for plasmid DNA that was isolated by polymerase chain reaction. The resultant fragments were sequence by dye terminator sequencing.

Protein-Protein Interaction Analysis—Fragments of PSTPIP and

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[1] The abbreviations used are: PTP, protein-tyrosine phosphatase; WASP, Wiskott-Aldrich syndrome protein; GST, glutathione *S*-transferase; GFP, green fluorescent protein; CHO, Chinese hamster ovary; BTK, Bruton's tyrosine kinase; aa, amino acids; HSCF, hematopoietic stem cell fraction.

WASP were subcloned by either the polymerase chain reaction or by restriction enzyme digestion. These fragments were incorporated either into a plasmid encoding the glutathione *S*-transferase (GST) protein, the green fluorescent protein (GFP), or an N-terminal Express™ epitope tag. The resultant plasmids were transfected into *Escherichia coli* (GST), COS, or CHO cells (GFP and Express™) and were analyzed as described previously (1, 2). Coprecipitation experiments were performed as described previously (1, 2).

Confocal Microscopy—Constructs expressing either GFP (WASP) or FLAG™ epitope-tagged (PSTPIP) proteins were co-transfected into CHO cells grown in glass chamber slides as described previously (1, 2). Transfected cells were than stained with either rhodamine phalloidin (for F actin) or Cy-3-labeled anti-FLAG™ (for PSTPIP) and were visualized using laser-scanning confocal microscopy.

PSTPIP Mutagenesis—Full-length PSTPIP or its SH3 domain was mutagenized by polymerase chain reaction-directed mutagenesis as described previously (1, 2). Tyrosine phosphorylation was analyzed by either co-transfection with a plasmid encoding v-Src or incubation of transfected cells with the tyrosine phosphatase inhibitor pervanadate as described previously (1, 2).

RESULTS AND DISCUSSION

Characterization of the PSTPIP Interaction with WASP—Screening of a murine BaF3 library using the yeast two-hybrid system with murine PSTPIP as bait resulted in the isolation of a murine homologue of WASP. Analysis of this interaction by yeast two-hybrid assays revealed that the full-length form of PSTPIP interacted *in vivo* with murine and human WASP, but a form of the protein lacking the SH3 domain did not (data not shown). These results suggested that the SH3 domain of PSTPIP was responsible for the original yeast two-hybrid interaction by recognition of a proline-rich motif in WASP (20–22). To test this hypothesis, full-length and deleted forms of WASP were expressed in COS cells, and the proteins were analyzed for interaction with the SH3 domain of PSTPIP expressed as a GST fusion. As Fig. 1 illustrates, two regions of WASP corresponding to residues 1–321 (WASP 5) and 321–502 (WASP 4) were able to interact with the GST SH3 fusion of PSTPIP, although the full-length protein (WASP FL) containing both of these regions appeared to bind significantly better. The proline-rich regions within WASP 4 and WASP 5 appeared to be required for this interaction, because WASP 1 (deleted for the proline-rich domain) did not interact. The binding sites were mapped to a finer level using oligopeptides derived from each of the proline-rich regions of WASP. Fig. 1 illustrates that two overlapping oligopeptides corresponding to residues 350–384 (peptides j and k) within the second binding site completely blocked the interaction *in vitro* at relatively low peptide concentrations, whereas another peptide derived from the other binding site (residues 302–325, peptide f) was able to partially inhibit this interaction at higher peptide concentrations. Although both of these regions contain stretches of prolines, Fig. 1 shows that polyproline is able to only weakly inhibit the interaction *in vitro* at relatively high concentrations. However, short stretches of proline were not alone sufficient to block the interaction, because such regions are found in other WASP-derived peptides tested (Fig. 1). Thus, the interaction between the PSTPIP SH3 domain and WASP is mediated by polyproline-containing regions that differ significantly from previously described SH3 recognition sites.

To examine the interaction between these two proteins *in vivo*, co-transfection studies were performed. Fig. 2*A* shows that PSTPIP was co-immunoprecipitated with WASP, demonstrating a physical interaction between the two proteins *in vivo*. Overexpression of WASP resulted in bundling of F actin and aggregation of WASP-F-actin complexes around the nucleus (11), and these data are replicated in Fig. 2*B* using WASP with the green fluorescent protein at the N terminus (GFP-WASP). This figure also illustrates a dose-dependent decrease

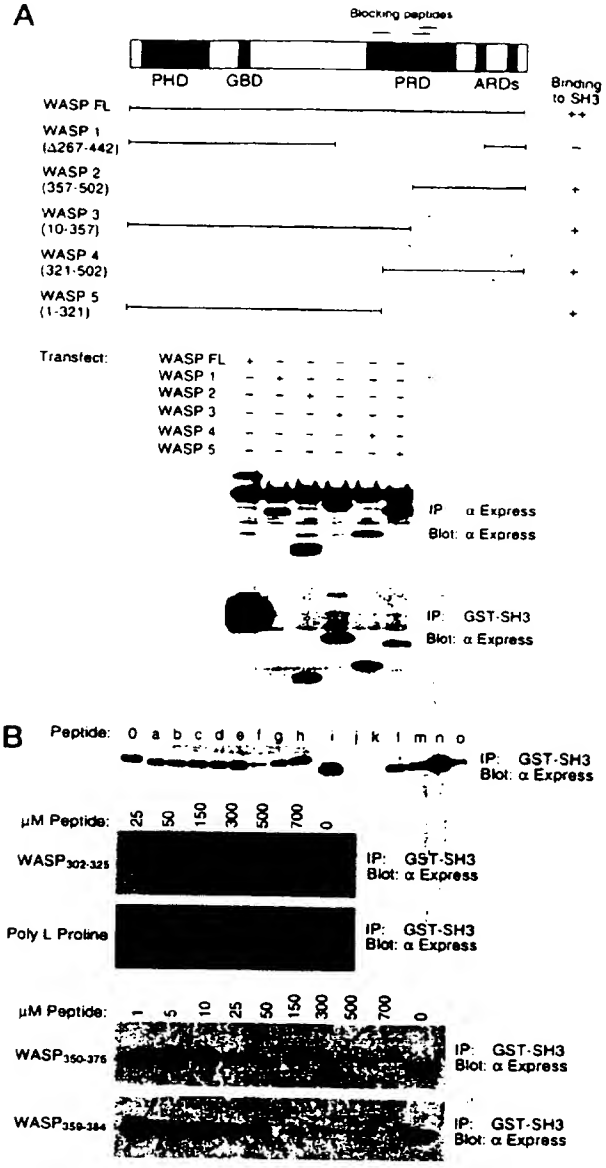


FIG. 1. **Mapping of the interaction between PSTPIP and WASP.** *A*, the domain structure of WASP is shown at the *top* the figure (7). *PHD*, pleckstrin homology domain; *GBD*, GTPase-binding domain; *PRD*, proline-rich domain; *ARDs*, actin regulatory domains; *IP*, immunoprecipitation. Various constructs used to map the WASP-PSTPIP interaction are also shown. The first blot (*IP: α Express* and *Blot: α Express*) illustrates that all of the constructs were expressed. The second blot (*IP: GST-SH3* and *Blot: α Express*) illustrates that WASP FL, WASP 2, WASP 3, WASP 4, and WASP 5 interacted with GST-PSTPIP SH3 domain, whereas WASP 2 did not. GST alone did not interact with any WASP-derived protein (data not shown). *B*, peptides derived from various proline-rich regions of WASP were tested for blocking the WASP-GST-SH3 interaction at 700 μM. Note that several WASP peptides that did not block at this high concentration contain extended stretches of proline residues: *a*, aa 317–340, PPPSRGGNQLPRPPIVGGNKGRSG; *b*, aa 331–354, IVGGNKGRSGPLPPVPLGIAPPPP; *c*, aa 370–393, PPPPATGRSGPLPPPPPGAGGPPM; *d*, aa 382–405, PPPPPGAGGPPMPPPPPPPPPPPS; *e*, aa 400–427, PPPPPSSGNGPAPPPLPPALVPAGGLAP; *f*, aa 302–325, AVRQEMRRQEPLPPPPPPSRGGNQ; *g*, aa 152–167, QSGDRRQLPPPPTPAN; *h*, aa 169–187, ERRGGLPPLPHPGGDQGG; *i*, aa 208–223, TSSRYRGLPAPGPSPA; *j*, aa 350–375, APPPPTPRGPPPPGRGGPPPPPPPAT; *k*, aa 359–384, PPPPGRGGPPPPPPPATGRSGPLPPP; *l*, aa 387–412, GAGGPPMPPPPPPPPPPPSSGNGPA; *m*, aa 341–366, PLPPVPLGIAPPPPTPRGPPPPGRGG; *n*, aa 178–201, PLHPGGDQGGPPVGPLSLGLATVD; *o*, poly L proline. Also illustrated are blocking titrations of the WASP interaction with the PSTPIP SH3 domain with inhibitory peptides *f*, *j*, *k*, and *o*.

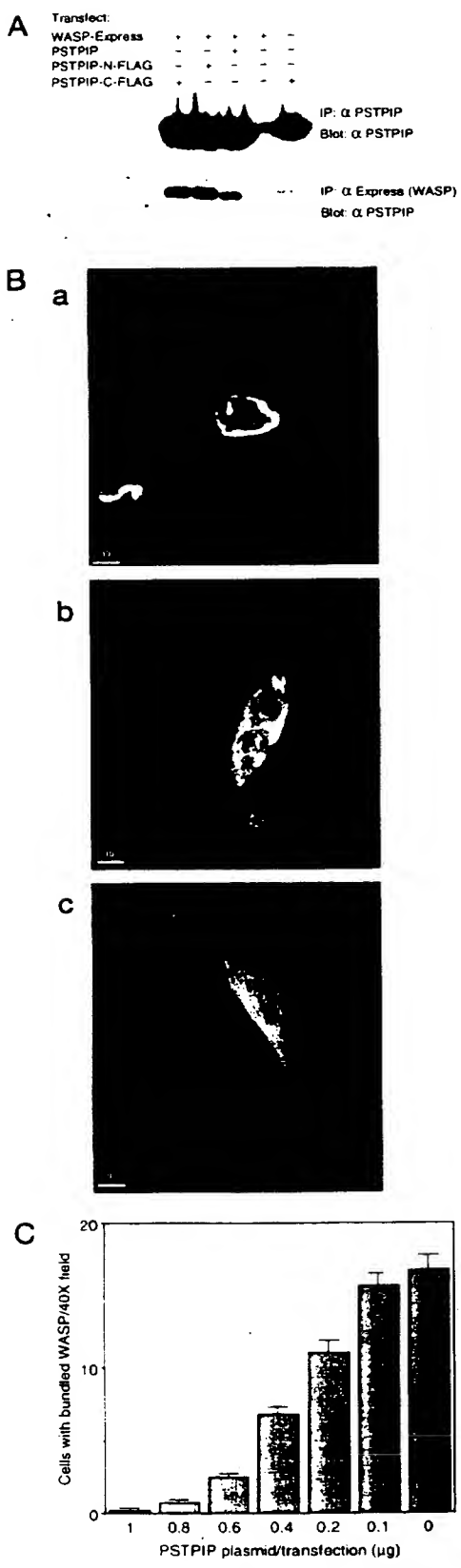


FIG. 2. **Physical interaction between WASP and PSTPIP in transfected cells and effects of PSTPIP on WASP bundling activity.** *A*, COS cells were transfected with Express® epitope-tagged WASP and either untagged PSTPIP or PSTPIP with a FLAG epitope tag at the N or C terminus. Lysates were immunoprecipitated with either polyclonal anti-PSTPIP antibody or anti-Express® tag monoclonal antibody and analyzed by Western blotting with horseradish peroxidase-conjugated anti-PSTPIP polyclonal antibody. *IP*, immunoprecipitation. *B*, a form of human wasp was constructed containing the green fluorescent protein at its N terminus (GFP-WASP). *a*, CHO cells were transfected with GFP-WASP (*green*) and stained with Cy-3-labeled phalloidin (*red*) to visualize F actin. Cells expressing WASP were visualized by confocal microscopy. As this micrograph illustrates, WASP and actin co-localize as compact bundles around the nuclei of cells expressing high levels of GFP-WASP, as has been previously described with an epitope-tagged version of WASP (11). *b*, cells were co-transfected with equivalent amounts of plasmids encoding GFP-WASP and PSTPIP with a FLAG epitope tag at the C terminus. Transfected cells were stained with Cy-3-labeled anti-FLAG antibody and visualized by confocal microscopy. WASP (*green*) and PSTPIP (*red*) exactly co-localize in these cells to give predominantly *yellow* filamentous staining throughout the cell. Note also that the GFP-WASP no longer bundles tightly around the nucleus but is now more diffusely spread throughout the cytoplasm. *c*, cells were co-transfected with equivalent amounts of plasmids encoding GFP-WASP and FLAG-tagged PSTPIP lacking the C-terminal SH3 domain (1). Transfected cells were stained with Cy-3-labeled anti-FLAG antibody and visualized by confocal microscopy. Note that the *red-stained* PSTPIP is now found to be diffusely spread throughout the cell, and there is now a lack of complete co-localization with GFP-WASP as was observed in *panel b*. *C*, cells expressing high levels of GFP-WASP were transfected with increasing quantities of a plasmid encoding PSTPIP, and the transfected cells were visualized by fluorescence microscopy. Cells with tightly bundled WASP were counted in each 40× field (10 fields for each plasmid concentration, ± S.E.).

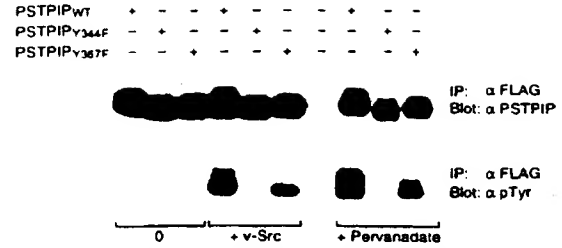
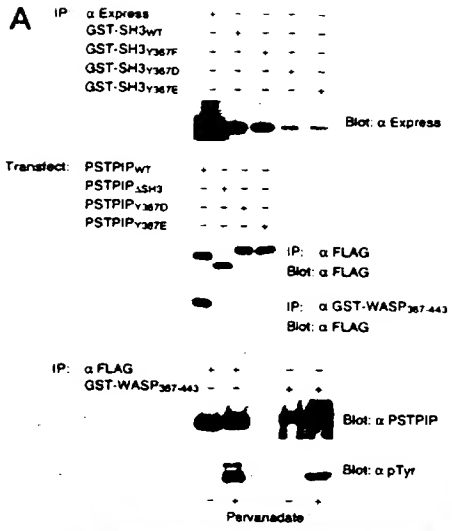


FIG. 3. **Identification phosphorylated tyrosine residues in PSTPIP.** Each tyrosine in murine PSTPIP was mutated to phenylalanine, and the mutants were analyzed either by transfection in the presence of v-Src tyrosine kinase or by incubation with the PTP inhibitor pervanadate. Cell lysates were immunoprecipitated with anti-FLAG antibody and analyzed for mobility shifts and phosphotyrosine content by Western blotting. The *upper gel* shows the reaction of the blots with anti-PSTPIP polyclonal antibody to demonstrate equal expression of all the constructs, whereas the *bottom blot* shows reactivity with anti-phosphotyrosine antibody. As illustrated here, mutation of tyrosine 367 to phenylalanine resulted in the disappearance of the slower migrating tyrosine phosphorylated bands, whereas mutation of tyrosine 344 resulted in a complete loss of tyrosine phosphorylation. *IP*, immunoprecipitation.

in this F-actin bundling activity by co-transfection of GFP-WASP overexpressing cells with increasing amounts of a plasmid expressing PSTPIP (Fig. 2*C*). This loss of actin bundling activity is similar to that observed when WASP is co-transfected with a dominant negative form of the small GTPase, CDC42 (11). Analysis of transfected cells for the cellular localization of GFP-WASP and PSTPIP revealed that these two proteins exactly co-localized and that the GFP-WASP was no longer tightly aggregated around the nucleus (Fig. 2*B*). PSTPIP is localized predominantly to the cortical cytoskeleton when expressed alone (1, 2), whereas co-expression with WASP appeared to reorient both PSTPIP and WASP to filamentous structures within the cytoplasm. As expected, co-localization required the C-terminal SH3 domain of PSTPIP, because co-transfection of GFP-WASP overexpressing cells with a form of PSTPIP lacking the SH3 domain (Fig. 2*B*) abolished the co-localization of the proteins, with the PSTPIP protein showing enhanced staining near the cell cortex and the GFP-WASP showing a diffuse staining throughout the cytoplasm. Thus,



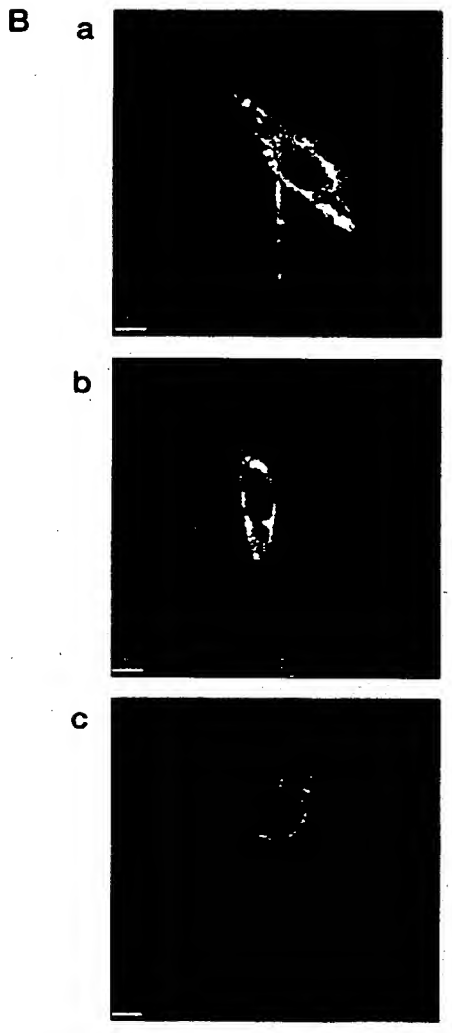


FIG. 4. **Loss of WASP-PSTPIP interaction by modification of tyrosine residue 367.** *A*, the *top panel* illustrates the precipitation of WASP with an N-terminal Express™ epitope tag using various forms of the PSTPIP SH3 domain. As can be seen, the anti-Express™ antibody, GST-wild type SH3, and GST-Y367F SH3 mutant all efficiently precipitate WASP, whereas incorporation of a negatively-charged aspartate or glutamate residue at position 367 results in an inhibition of WASP precipitation by these mutant GST-SH3 proteins. The *middle panel* illustrates that a GST fusion derived from the WASP polyproline-rich domain (*GST WASP*$_{367-443}$) efficiently precipitates the wild type form of PSTPIP but cannot interact with the SH3 deleted form nor with the forms of PSTPIP containing aspartate or glutamate residues at position 367. The *bottom panel* illustrates that the slower migrating phosphotyrosine forms of PSTPIP, corresponding to PSTPIP phosphorylated at both tyrosines 344 and 367, do not react efficiently with the GST WASP$^{367-443}$ protein, suggesting that phosphorylation at residue 367 inhibits the binding of the SH3 domain to the WASP proline-rich region. *B*, micrograph *a* illustrates the co-transfection of GFP-WASP with wild type PSTPIP with a C-terminal FLAG epitope tag. Transfected cells were stained with Cy-3-labeled anti-FLAG and were visualized by confocal microscopy. Again, note the complete co-localization of GFP-WASP and PSTPIP (*yellow* staining only). Micrographs *b* and *c* show similar co-transfections performed with GFP-WASP and PSTPIP mutants with either an aspartate (*b*) or a glutamate (*c*) at position 367. Note that the *red-stained* PSTPIP now becomes diffuse throughout the cytoplasm and is not co-localized with GFP-WASP.

interactions between the PSTPIP SH3 domain and proline-rich regions of WASP mediate co-localization of the two proteins *in vivo*. Furthermore, they are consistent with functional modulation of WASP actin bundling activity by PSTPIP.

Mapping the Phosphorylated Tyrosines in PSTPIP—PSTPIP was previously shown to be tyrosine phosphorylated in cells either expressing the v-Src tyrosine kinase or incubated with the PTP inhibitor, pervanadate (1). To analyze a possible regulatory role of tyrosine phosphorylation in the interactions between PSTPIP and WASP, each tyrosine in PSTPIP was individually mutated to phenylalanine. The mutants were then analyzed for phosphotyrosine content and mobility shifts by SDS-polyacrylamide gel electrophoresis and immunoblotting with an anti-phosphotyrosine antibody after either co-transfection of v-Src tyrosine kinase or incubation of transfected cells in the presence of pervanadate. Fig. 3 illustrates that mutation of two tyrosines of murine PSTPIP, at positions 344 and 367, to phenylalanine resulted in modifications of PSTPIP tyrosine phosphorylation in both v-Src-transfected and pervanadate-treated cells, whereas mutation of the other tyrosines to phenylalanine showed no change in banding patterns or phosphotyrosine content (data not shown). Mutation of tyrosine residue 367 alone resulted in the disappearance of the slower migrating phosphotyrosine forms of PSTPIP under both phosphorylation conditions, suggesting that the slower migrating bands are due to phosphorylation of tyrosine 367. Mutation of tyrosine 344 alone resulted in a complete loss of phosophotyrosine in the protein (Fig. 3). This result implied that phosphorylation of tyrosine 344 was required for the subsequent phosphorylation of tyrosine residue 367. In addition, the data imply that the residual single tyrosine phosphorylated band in the form of PSTPIP mutated at position 367 is due to tyrosine residue 344, whereas the more slowly migrating forms are due to phosphorylation at both the 344 and 367 positions. Examination of the sequence surrounding tyrosine 344 revealed that it is a significant consensus site for modification by Src family member tyrosine kinases (23),[2] consistent with the possibility that tyrosine residue 344 is directly phosphorylated by either transfected v-Src or by an endogenous Src family tyrosine kinase that is activated by pervanadate treatment. Tyrosine residue 367 is within the polyproline recognition site of the SH3 domain of PSTPIP (20–22), and structure-function analyses of the SH3 domains in several other proteins has shown that this tyrosine is involved with binding of the proline-rich ligand. These data thus suggest that PSTPIP is sequentially phosphorylated, first at residue 344 by a Src family member tyrosine kinase and subsequently at a tyrosine within the SH3 polyproline-binding site.

Modification of Tyr^{367} *Modulates the PSTPIP Interaction*

[2] L. Cantley, personnel communication.

...ith WASP—The above data suggested that binding of PSTPIP ...o WASP might be regulated by phosphorylation of tyrosine 367 ...n the PSTPIP SH3 domain, because this residue is found ...thin the polyproline-binding pocket (20–22). To test this ...sibility, tyrosine 367 was mutated to either an aspartate or ...lutamate to mimic the incorporation of the negatively ...rged phosphate group at this position. Structural analysis ...other SH3 domains (20–22) suggests that this tyrosine is ...olvent exposed and does not interact with other residues, ...uggesting that these mutations should not globally affect the ...tructure of the PSTPIP SH3 domain. GST fusion proteins ...ontaining these mutations were tested for their ability to ...nteract with WASP in an *in vitro* binding assay. In addition, a ...GST fusion containing the second proline-rich PSTPIP-binding ...te of WASP (residues 357–443) was also tested for its ability ... bind to mutated forms of PSTPIP. As Fig. 4 illustrates, ...ither the two mutants containing acidic residues at the ty...osine 367 position nor the form of PSTPIP missing the SH3 ...domain were able to interact strongly with WASP, whereas ...both the wild type SH3 motif as well as an SH3 domain with ...phenylalanine substituted at position 367 were bound to ...WASP, suggesting that the incorporation of an acidic side chain ...at this site negatively regulates the interaction between PST...PIP and WASP. This result suggests that the incorporation of ...a negatively charged phosphate at position 367 would inhibit ...PSTPIP-WASP binding and that the SH3 domain interacts ...with WASP as predicted from previous structure-function stud...ies (20–22). To examine the effects of these mutations *in vivo*, ...co-transfection experiments were performed in conjunction ...with confocal microscopy. Fig. 4 illustrates that although wild ...type PSTPIP was able to co-localize with WASP *in vivo*, PST...PIP with either the aspartate or glutamate mutations at posi...tion 367 showed the same lack of co-localized staining as was ...found for the form of the protein that was lacking the SH3 ...domain (Fig. 2). Finally, Fig. 4 illustrates that a GST fusion ...protein containing the second proline-rich PSTPIP-binding site ...of WASP is able to interact only with the more rapidly migrat...ing tyrosine phosphorylated form of PSTPIP corresponding to ...tyrosine 344 and does not precipitate the slower migrating ...bands corresponding to PSTPIP tyrosine phosphorylated at ...both positions 344 and 367. These data suggest that phospho...rylation of tyrosine 367 in the SH3 domain of PSTPIP modu...lates the interaction between this cytoskeletal-associated pro...tein and WASP.

Individuals with Wiskott-Aldrich syndrome have deficits in their hematopoietic cells, and these deficiencies appear to be due to cytoskeletal disruptions (10, 24, 25). Persons with mutations in WASP have defects in lymphocyte surface microvilli and platelet morphology that manifest themselves as immunodeficiency and thrombocytopenia, respectively (6). WASP is involved with modulation of the cytoskeleton by virtue of its ability to mediate actin bundling in transfected cells (11), and a yeast homologue is required both for maintenance of the cortical cytoskeleton as well as cytokinesis (13, 14). WASP has also been shown to interact both with CDC42, a small GTPase that affects the cytoskeleton and modulates WASP actin bundling activity, as well as tyrosine kinases and the Nck and Grb2 adaptor proteins (11, 12, 15–19). The data presented suggest that the interaction between PSTPIP and WASP modulates actin assembly, and this binding event is regulated by tyrosine phosphorylation of the PSTPIP SH3 domain. PSTPIP is tightly bound to PTP HSCF *in vivo*, and in a nonsynchronized population of cells, this interaction appears to maintain PSTPIP in a state predominantly lacking phosphorylated tyrosine (1, 2). This would allow for a constitutive association between PSTPIP and WASP, as is observed here in transfected cells expressing both proteins. Tyrosine phosphorylation of the SH3 domain of PSTPIP would be likely to release WASP from the PSTPIP-PTP HSCF complex, thus allowing, for example, for an activation of WASP actin modulatory activity or migration of the protein to a different region of the cell. Alternatively, this same phosphorylation event would release PSTPIP from the grasp of WASP, which might allow for the movement of PSTPIP to other cellular domains, such as the cleavage furrow (1, 3). At least one other SH3-containing protein, the Bruton's tyrosine kinase (BTK), contains an autophosphorylated tyrosine at a site comparable with that found in the PSTPIP SH3 domain (26), and both BTK and ITK, an SH3 domain-containing tyrosine kinase related to BTK, are known to bind to WASP via an SH3-polyproline interaction (15, 16, 27). These data provide evidence for the control of the cytoskeleton by modulation of the tyrosine phosphorylation of an SH3 domain, and they suggest that other SH3-polyproline interactions may be similarly regulated. Because many cytoskeletal regulatory proteins contain SH3 domains, these data suggest a general mechanism for the control of the cytoskeleton via the interaction of these domains with the diversity of proline-rich proteins including N-WASP, the formins, VASP, and Enabled (28–31). Future studies directed toward an analysis of the role of the WASP-PSTPIP-PTP HSCF complex in the control of the cytoskeleton during interphase and cytokinesis should provide significant insights into the regulation of cell shape and division by tyrosine phosphorylation.

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